International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Therapeutic & Diagnostic Applications of Biosurfactants of Microbial Origin

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Abstract
Biosurfactants, amphipathic molecules predominantly produced by microorganisms, represent a sustainable and versatile alternative to conventional synthetic surfactants. Their unique physicochemical properties, including exceptional surface and interfacial tension reduction, emulsification, and foam stabilisation capabilities, are complemented by significant environmental advantages such as low toxicity, high biodegradability, and production from renewable resources. This review highlights microbial biosurfactant production, detailing key producers, their biosynthesis pathways, and advancements in fermentation and genetic engineering strategies for enhancing yields and altering molecular structures. Furthermore, it examines the critical downstream processing methods for biosurfactant isolation and purification, highlighting both their efficacy and inherent challenges. A significant focus is placed on the diverse and expanding medical and health applications of biosurfactants, particularly their potent antimicrobial and anti-biofilm activities, and innovative roles in drug delivery systems, and their promising immunomodulatory, diagnostic, and therapeutic potentials. While the high production and purification costs currently impede widespread commercialisation, ongoing advancements in biotechnology and process optimisation underscore a future where biosurfactants play an increasingly indispensable role in sustainable healthcare solutions.

Keywords: Amphipathic molecules, Biosurfactants, Emulsions, Microbial production, Medical applications

1. Introduction to Biosurfactants

1.1. Definition, Amphiphilic Nature, and General Properties

Biosurfactants are a class of amphipathic molecules synthesised by various living organisms, including plants, animals, and most notably, microorganisms.[1] Their defining characteristic is their ability to significantly reduce surface and interfacial tensions, thereby facilitating the mixing of otherwise immiscible substances.[1] This unique functionality stems from their dual molecular architecture, comprising both hydrophilic (water-loving) and hydrophobic (water-repelling) components.[2]

The hydrophilic region of a biosurfactant molecule can be composed of diverse chemical groups such as carbohydrates, amino acids, cyclic protein peptides, phosphates, or alcohols. Conversely, the hydrophobic region typically consists of long-chain fatty acids, hydroxyl fatty acids, or α-alkyl-β-hydroxy fatty acids, often ranging in length from 8 to 18 carbon atoms.[3] This amphiphilic nature allows biosurfactants to

spontaneously assemble at interfaces, such as air-water or water-oil boundaries, effectively lowering the interfacial forces.[2]

The key properties that render biosurfactants highly valuable include:

- **Surface and Interfacial Tension Reduction:** This is a primary function, enabling them to dramatically lower the surface tension of water, for instance, from approximately 72 mN/m to values as low as 27-35 mN/m. Similarly, they can reduce interfacial tension between oil and water to remarkably low levels, such as 0.32-3.79 mN/m. Surfactin, a prominent biosurfactant, is particularly noted for its exceptional surface activity.[3]
- **Emulsion Formation and Stabilization:** Biosurfactants are highly effective in creating and stabilizing emulsions by forming a protective layer around dispersed droplets, which prevents their coalescence and phase separation.[3]
- **Foam Stabilization:** They contribute to the stability of foams by reducing the surface tension at the liquid-air interface within foam bubbles, thereby maintaining the foam's structural integrity.[3]
- **Interfacial Activity:** Beyond liquid-liquid and liquid-gas interfaces, biosurfactants can also modify the surface properties of solid materials, enhancing their wetting or spreading characteristics.[3]

The fundamental amphiphilic structure of biosurfactants directly enables their versatile surface-active properties. This inherent molecular design allows them to interact effectively at various interfaces, leading to their capacity for tension reduction, emulsification, and foam stabilization. These physicochemical attributes, coupled with their inherent advantages over synthetic counterparts, position biosurfactants as compelling and sustainable alternatives across numerous industries[4]. Compared to chemically synthesized surfactants, biosurfactants offer significant benefits, including lower toxicity, high environmental compatibility, ready biodegradability, and their production from renewable raw materials.[2] Furthermore, they exhibit stable activity across a wide range of environmental conditions, such as varying pH, salinity, and temperature, and can be highly selective and effective even at low concentrations.[5-10] This combination of versatile properties and environmental compatibility explains their expanding application scope, particularly in sensitive sectors like medicine and health.

1.2. Classification and Structural Diversity of Microbial Biosurfactants

Biosurfactants display remarkable structural and functional diversity, leading to their classification based on several criteria.[2] This extensive diversity is a critical determinant of their functional specificity. Different structural classes exhibit distinct properties, making them suitable for varied applications.

Classification by Molecular Weight:

- **Low Molecular Weight (LMW):** These biosurfactants typically have molecular weights ranging from 200 to 1000 Daltons. This group includes glycolipids, lipopeptides, and phospholipids. They are primarily recognized for their efficiency in lowering surface and interfacial tension.[11]
- **High Molecular Weight (HMW):** This category encompasses biosurfactants with molecular weights generally exceeding 1000 Daltons. Examples include lipopolysaccharides, polysaccharide-based biosurfactants, and other polymeric compounds. HMW biosurfactants are typically more effective as stabilizing agents for emulsions and can exhibit diverse functionalities beyond surface activity, such as immunomodulatory effects and biofilm regulation.[3]

Classification by Chemical Composition:

- **Glycolipids:** These biosurfactants consist of a sugar moiety covalently linked to a lipid chain. Key examples include rhamnolipids, sophorolipids, trehalolipids, and mannosylerythritol lipids (MELs).[3]
- **Lipopeptides:** Characterized by a peptide (protein) chain linked to a lipid (fatty acid) chain. Surfactin

and iturin are well-known representatives of this class.[12]

- **Phospholipids:** Composed of a phosphate group, a glycerol backbone, and fatty acid chains. These are commonly found as major components of cell membranes. Phosphatidylethanolamine is a notable example that can function as a biosurfactant.[2]
- **Polymeric Biosurfactants:** This class comprises large macromolecules with repeating monomeric units, often complex mixtures of polysaccharides, proteins, or lipopolysaccharides. Emulsan and liposan are prominent examples within this category.[13]
- **Particulate Biosurfactants:** These involve extracellular membrane vesicles that play a crucial role in forming microemulsions, which can significantly impact alkane uptake in microbial cells.[3]

The extensive classification of biosurfactants by molecular weight and chemical composition highlights their remarkable structural variability.[14] This diversity is not merely a descriptive characteristic but a fundamental aspect that dictates their functional specificity. For instance, LMW biosurfactants like glycolipids and lipopeptides are highly effective at reducing surface tension, making them ideal for applications requiring emulsification and wetting. In contrast, HMW polymeric biosurfactants excel as emulsion stabilizers and can exhibit complex biological effects such as immunomodulation.[15] This direct correlation between structural variations and distinct functional properties underscores that the selection or engineering of a biosurfactant for a particular application necessitates a thorough understanding of its chemical class and specific structural nuances. This principle guides targeted research and development efforts to create biosurfactants optimised for precise industrial and medical uses.

2. Microbial Production of Biosurfactants

Biosurfactants are naturally synthesised and secreted by a diverse array of microorganisms, including bacteria, fungi, yeasts, and actinomycetes, as integral components of their growth and metabolic processes.[2] These molecules can either remain associated with the microbial cell surface or be released extracellularly into the culture medium.[16]

Table 1: Major Classes of Biosurfactants, Their Chemical Structures, and Key Properties

Class	Examples	Structural Components	Key Properties/Function
Glycolipids	Rhamnolipids	Rhamnose sugar(s) + 3-(hydroxyalkanoyloxy)alkanoic acid (HAA) fatty acid	Surface tension reduction, emulsification, antimicrobial, anti-biofilm
	Sophorolipids	Sophorose (glucose disaccharide) + 16-18 carbon hydroxy fatty acid	Surface tension reduction, antimicrobial, foaming, emulsification

	Mannosylerythritol Lipids (MELs)	Mannose + erythritol residue + two fatty acids chains; variable acetylation	Surface activity, cell differentiation, protein/antibody interaction, skin care
Lipopeptides	Surfactin	Cyclic heptapeptide + β-hydroxy fatty acid	Exceptional surface activity, antimicrobial, anti-biofilm, anticancer, hemolytic
	Iturin	Cyclic peptide (seven amino acid residues) + 11-12 carbon fatty acid chain	Antimicrobial (especially antifungal), surface activity
Phospholipids	Phosphatidylethanolamine	Glycerol + two fatty acids + phosphate group + ethanolamine	Emulsification, solubilization, membrane interaction, immunomodulatory
Polymeric Biosurfactants	Emulsan	Polysaccharide backbone (aminosugars: D-galactosamine, D-galactosaminouronic acid, dideoxydiaminohexose) + O-acyl/N-acyl fatty acids	Emulsion stabilization, immunomodulatory, biofilm regulation
	Liposan	Heteropolysaccharide (glucose, galactose, galactosamine, galacturonic acid) + protein	Emulsification, water-soluble, foam stabilization

2.1. Key Microbial Producers and Biosynthesis Pathways

The microbial production of biosurfactants involves complex biochemical pathways, often leading to a variety of structural analogues depending on the microbial strain and environmental conditions.[17-34]

2.1.1. Glycolipids

Rhamnolipids:

Rhamnolipids are a well-studied class of glycolipid biosurfactants primarily produced by Gram-negative *Pseudomonas* species, with *Pseudomonas aeruginosa* being the most prominent producer.[3, 18] Structurally, rhamnolipids consist of one or two rhamnose sugar units, which form the hydrophilic head, linked to a 3-

(hydroxyalkanoyloxy) alkanoic acid (HAA) fatty acid tail, serving as the hydrophobic component. Common variants include mono-rhamnolipids and di-rhamnolipids, with Rha-Rha-C10-C10 being a frequently reported homologue (Figure 1).[31] The precise chemical structure of rhamnolipids can vary significantly depending on the specific bacterial strain, the carbon source used, and the prevailing culture conditions.[33]

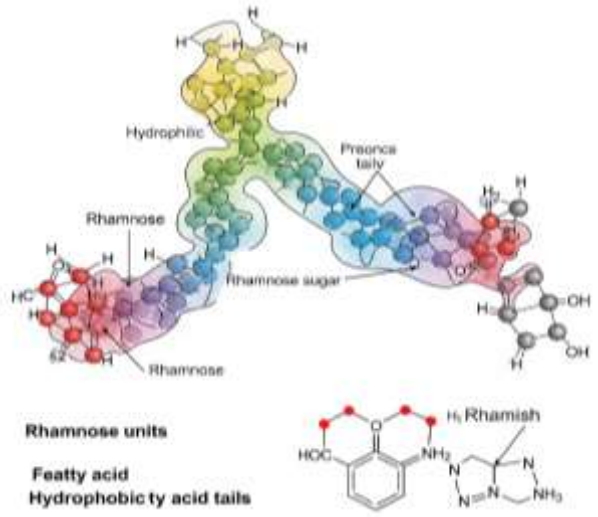

Figure 1: Three Dimensional Diagram of Microbial Rhamnolipids

The biosynthesis of rhamnolipids proceeds through a series of enzymatic steps. It begins with the transfer of TDP-L-rhamnose. The RhlA enzyme is responsible for synthesizing the 3-(3-hydroxyalkanoyloxy) alkanoic acid (HAA) precursor.[35] This HAA is then converted into mono-rhamnolipid by the action of the RhlB enzyme. Subsequently, di-rhamnolipids are formed from mono-rhamnolipids through a third reaction catalysed by the RhlC enzyme.[35] This intricate pathway is tightly regulated by quorum sensing (QS) systems, which involve diffusible signalling molecules such as PAI-1 and PAI-2, and activators like LasR and RhlR. This regulation is particularly pronounced at high bacterial cell densities, illustrating a coordinated production effort within the microbial population.[36] The significant role of quorum sensing in regulating rhamnolipid biosynthesis indicates a broader biological principle where microorganisms coordinate the production of secondary metabolites like biosurfactants in a population-density-dependent manner.[37] Understanding and manipulating these QS pathways through genetic engineering or environmental cues could be a powerful strategy for optimizing biosurfactant yields and controlling their production kinetics, leading to more efficient and cost-effective industrial processes.

Sophorolipids:

Sophorolipids are another important class of glycolipid biosurfactants, predominantly synthesized by non-pathogenic yeast species, most notably *Candida bombicola* (also known as *Starmerella bombicola*).[3] These molecules are composed of a sophorose disaccharide, a glucose-derived sugar characterised by an unusual β-1,2 bond, linked to a hydrophobic fatty acid tail, typically containing 16 or 18 carbon atoms. Sophorolipids exist in two main forms: acidic (where the carboxylic end is free) or lactonic (where the carboxylic end is internally esterified, usually at the 4″ position). They can also be acetylated at specific positions, such as the 6′- and/or 6″- positions.[39] It is observed that lactonic sophorolipids are generally more effective at reducing surface tension and exhibit superior antimicrobial activity, whereas acidic forms tend to possess better foaming properties.[40]

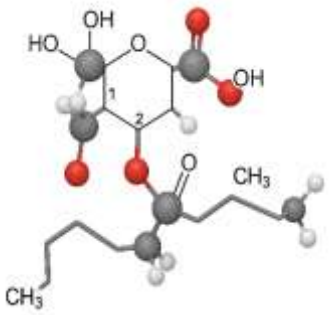

Figure 2: Three-Dimensional Structure of Microbial Sophorolipid with molecular details

The biosynthesis pathway for sophorolipids in *S. bombicola* involves five distinct steps. It commences with the (sub)terminal hydroxylation of a fatty acid, which can be derived from the substrate or synthesised *de novo*. This initial step is catalysed by a cytochrome P450 monooxygenase, specifically CYP52M1. Subsequent glycosylation steps, mediated by two UDP-glucosyltransferases (UGTA1 and UGTB1)[41], sequentially add glucose molecules to form an acidic sophorolipid. Further modification involves acetylation by an acetyltransferase (AT) and an extracellular lactonisation step catalysed by the *S. bombicola* lactone esterase (SBLE), which converts acidic sophorolipids into their lactonic forms.[38]

Mannosylerythritol Lipids (MELs):

Mannosylerythritol lipids (MELs) are glycolipid biosurfactants produced by fungi belonging to the Ustilaginaceae family, including Pseudozyma spp. and *Moesziomyces aphidis*.3 Their structure comprises a hydrophilic sugar core, specifically 4-O-β-D-mannopyranosyl-D-erythritol, and multiple hydrophobic residues. These hydrophobic components typically include two fatty acid chains, usually ranging from C2 to C18, which collectively sum to 18–22 carbon atoms. MELs are further classified into congeners (MEL-A, -B, -C, and -D) based on their acetylation pattern, which directly influences their polarity.[44] The use of unconventional fatty acids, such as ricinoleic acid from castor oil, can lead to the production of novel MEL structures that are more hydrophilic.[45] The production of MELs is governed by a gene cluster consisting of five essential genes:

emt1, mac1, mac2, mat1, and *mmf1*.[45]

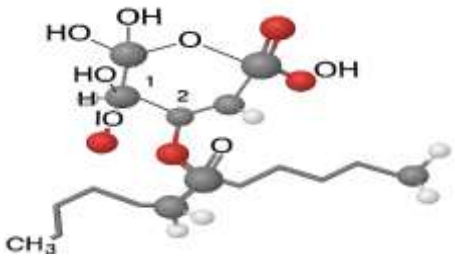

Figure 2: Three-Dimensional Structure of Microbial Mannosylerythritol Lipids

The detailed descriptions of rhamnolipid, sophorolipid, and MEL structures consistently highlight that their specific chemical configurations, such as fatty acid chain length, saturation, and acetylation patterns, are significantly influenced by the carbon source and culture conditions employed during microbial production.[29] This observation establishes a clear causal relationship: the choice of raw materials directly dictates the structural variants of the biosurfactants produced. This implies that by carefully selecting and manipulating substrates, researchers can precisely tailor biosurfactants with desired physicochemical

properties for specific applications, moving beyond a generic production approach. This is a critical consideration for optimizing biosurfactant performance in targeted industrial and medical uses.

2.1.2. Lipopeptides

Surfactin:

Surfactin is recognized as one of the most potent biosurfactants and is primarily produced by Bacillus subtilis and other *Bacillus* sp. Structurally, surfactin is a cyclic lipopeptide composed of a heptapeptide chain with a specific amino acid sequence (L-Glu-L-Leu-D-Leu-L-Val-L-Asp-D-Leu-L-Leu) linked to a β-hydroxy fatty acid chain, typically comprising 13–16 carbon atoms, via a lactone bond. This linkage forms a characteristic cyclic structure.[2] The inherent diversity in the amino acid sequence and the length of the fatty acid chain contributes to the existence of numerous surfactin variants, each with potentially distinct properties.[2]

The biosynthesis of surfactin relies on a multi-modular enzyme complex known as non-ribosomal peptide synthetase (NRPS) SrfA, which is encoded by the *srfAA-AD* operon.[47] A crucial enzyme in this process is the 4-phosphopantetheinyl transferase Sfp, which activates the NRPS, a necessary step for surfactin formation.[47] Surfactin biosynthesis is tightly regulated by complex molecular networks, including two quorum sensing systems (ComX pheromone and CSF) that mediate cell-cell communication, and the master regulator Spo0A, which plays an indispensable role in its proper synthesis.[47]

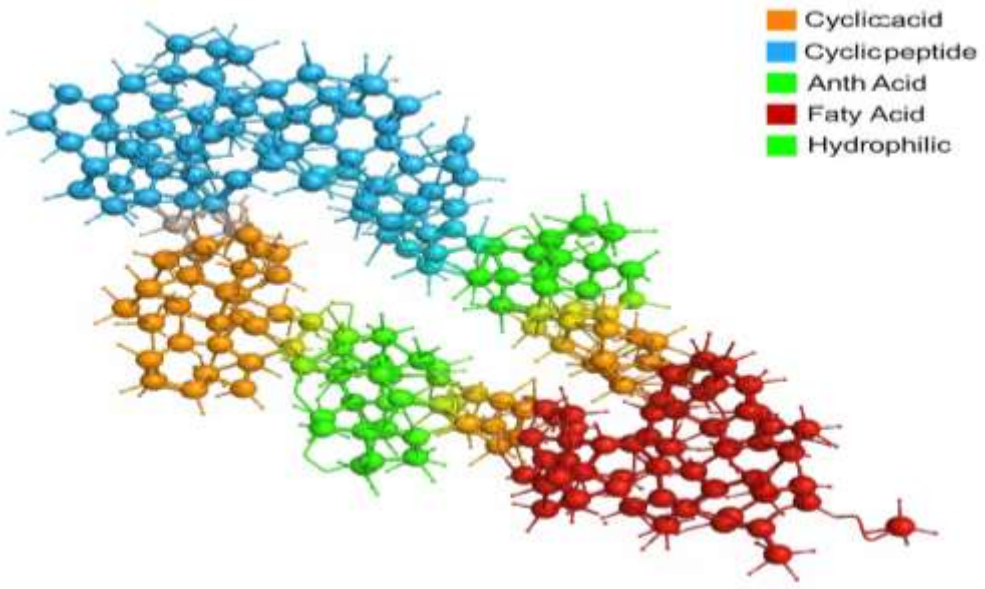

Figure 3: Spatial Diagram Representing Structure of Microbial Surfactin

The precursor molecules required for surfactin synthesis include branched-chain amino acids (such as leucine and valine) and various fatty acids.[47] The crucial role of quorum sensing in regulating surfactin production, similar to rhamnolipids, indicates a broader biological principle where microorganisms coordinate the production of secondary metabolites like biosurfactants in a population-density-dependent manner. Understanding and manipulating these QS pathways could be a powerful strategy for optimizing biosurfactant yields and controlling their production kinetics, leading to more efficient and cost-effective industrial processes.

2.1.3. Phospholipids

Phosphatidylethanolamine (PE):

Phosphatidylethanolamine (PE) is a class of phospholipids that can function as biosurfactants. Several bacteria, such as *Acinetobacter* sp. HOI-N[48] and *Rhodococcus erythropolis*,[49] as well as various yeast species, are known to produce significant quantities of phospholipids, particularly when grown on n-alkanes or other hydrocarbon substrates. PE is a glycerophospholipid composed of a glycerol backbone esterified with two fatty acids, a phosphate group, and an ethanolamine head group.3 The fatty acid chains typically range from 16 to 20 carbon atoms, with saturated fatty acids generally found at the sn-1 position and longer, often unsaturated, chains at the sn-2 position.[50] PE is characterized as a non-bilayer forming lipid, which means it can influence membrane curvature and fusion processes within biological membranes.[51]

In bacteria, PE can serve as a precursor for the synthesis of phosphatidylcholine (PC) via the N-methylation pathway. In this pathway, phospholipid N-methyltransferases (Pmts) catalyze sequential methylation reactions of the ethanolamine head group of PE, utilizing S-adenosyl-L-methionine (SAM) as a methyl donor.[52]

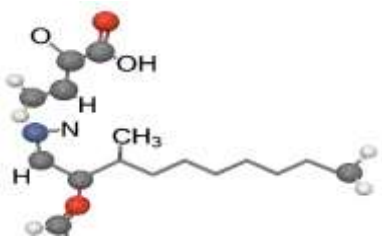

Figure 4: Three Dimensional of Microbial phosphatidylethanolamine (Glycerol Backbone, Phosphoethanolamine headgroup, Dual Fatty Acid Chains)

2.1.4. Polymeric Biosurfactants

Emulsan:

Emulsan is a notable anionic lipo-heteropolysaccharide and protein complex produced by *Acinetobacter calcoaceticus*, particularly strain RAG-1.[8.] Its structure consists of an unbranched polysaccharide backbone adorned with O-acyl and N-acyl bound fatty acid side chains. The polysaccharide backbone is characteristically composed of three amino-sugars D-galactosamine, D-galactosaminouronic acid, and a dideoxydiaminohexose in a 1:1:1 ratio. The fatty acid side chains vary in length from 10 to 22 carbons and can constitute a significant portion, up to 23%, of the polymer's total weight.[53] The amphipathic nature of emulsan, which gives it its emulsifying properties, arises from the synergistic combination of its hydrophilic anionic sugar units and hydrophobic fatty acid side groups.[54]

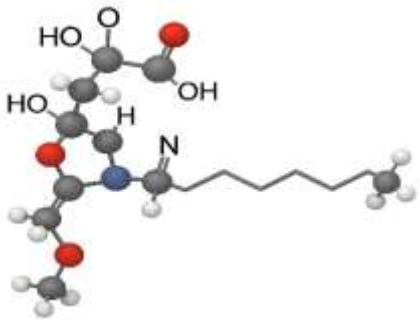

Figure 5: Three Dimensional of Microbial Emulsan

The biosynthesis of emulsan is primarily controlled by the *wee* gene cluster, which is responsible for the synthesis of both high molecular weight (HMW) and low molecular weight (LMW) polysaccharides.[35] The composition of the fatty acid side chains, a key determinant of emulsan's properties, can be manipulated by altering the culture conditions or through genetic engineering of the producing strain.[56-61]

Liposan:

Liposan is an extracellular, water-soluble emulsifier synthesised by yeast, most notably Candida lipolytica.13 This polymeric biosurfactant is composed of approximately 83% carbohydrate and 17% protein. The carbohydrate portion is a heteropolysaccharide consisting of glucose, galactose, galactosamine, and galacturonic acid.[22]

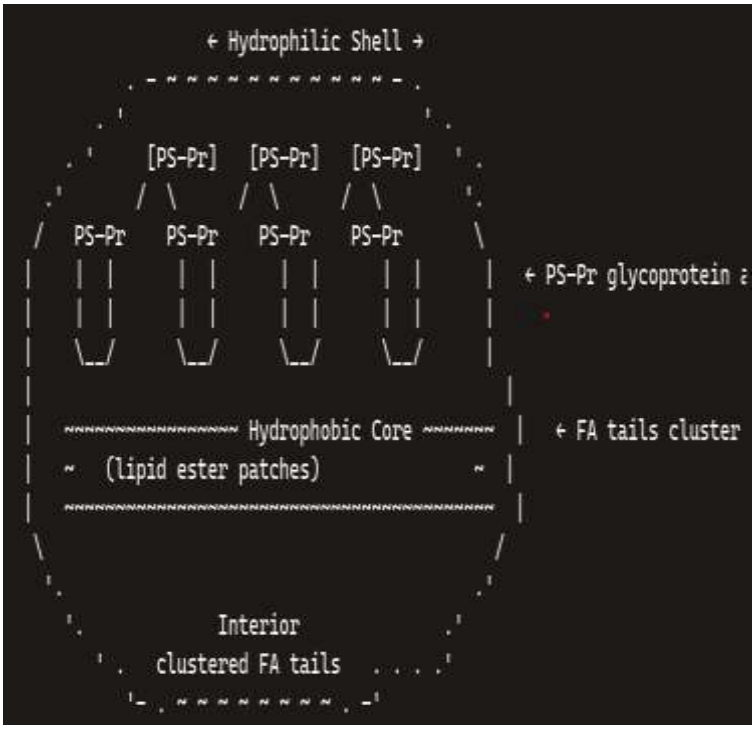

Figure 6: Three Dimensional of Microbial Liposan; PS = Polysaccharide monomer (e.g., galacturonic acid, mannose) • Pr = Protein moiety (glycoprotein linkage) • FA = Fatty-acid chains (C16–C18) • "~" = Hydrophobic lipid patches

Y. lipolytica is known for its remarkable ability to utilize a wide range of complex carbon sources, including both hydrophilic and hydrophobic substrates, and exhibits high tolerance to varying salt

concentrations and pH values.[62] Its lipolytic activity is crucial, enabling the hydrolysis of triglycerides into free fatty acids and glycerol at the oil-water interface within the culture medium. These liberated fatty acids are then actively transported into the cell, where they can undergo various enzymatic modifications, including the β-oxidation process producing liposan.[62]

2.2. Fermentation Strategies and Process Optimization

Optimizing the production of biosurfactants is crucial for their commercial viability, as their synthesis can be an expensive process.[30] This involves careful selection and control of fermentation techniques and process parameters.

Fermentation Techniques:

Biosurfactant production can be achieved using three primary fermentation processes:

Batch Fermentation: In this method, all necessary nutrients are supplied at the initiation of the fermentation process. The culture is then allowed to grow until the nutrients are depleted, at which point the broth is harvested. This technique is characterized by its simplicity and a low risk of external contamination, making it suitable for cultures that naturally achieve high yields and can tolerate high initial nutrient concentrations.[30]

Table 2: Notable Microbial Producers of Biosurfactants and Their Specific Products

Microbes	Biosurfactant Class	Specific Biosurfactant	Key Biosynthesis Genes/Enzymes	Key Structural Features
Pseudomonas aeruginosa	Glycolipid	Rhamnolipids	*rhlAB*, RhlC	Rhamnose sugar(s) + 3-(hydroxyalkanoyloxy)alkanoic acid (HAA) fatty acid
Bacillus subtilis	Lipopeptide	Surfactin	*srfA* operon, Sfp gene	Cyclic heptapeptide + β-hydroxy fatty acid
Candida bombicola (*Starmerella bombicola*)	Glycolipid	Sophorolipids	CYP52M1, UGTA1, UGTB1, AT, SBLE	Sophorose + 16-18 carbon hydroxy fatty acid (acidic/lactonic forms)
Pseudozyma spp.	Glycolipid	Mannosylerythritol Lipids (MELs)	*emt1, mac1, mac2, mat1, mmf1*	Mannose + erythritol + two fatty acids; variable acetylation
Acinetobacter calcoaceticus	Polymeric	Emulsan	*wee* gene cluster	Polysaccharide backbone (aminosugars) + O-acyl/N-acyl fatty acids

Candida lipolytica	Polymeric	Liposan	(Hydrolysis of triglycerides, β-oxidation)	Heteropolysaccharide (glucose, galactose, galactosamine, galacturonic acid) + protein
Rhodococcus erythropolis	Glycolipid	Trehalolipids	(Mycolic acid synthesis)	Trehalose units linked to mycolic acids

- **Fed-Batch Fermentation:** Similar to batch fermentation, fed-batch starts with a partial supply of nutrients. Additional nutrients are then incrementally added as the fermentation progresses. This strategy is employed to prevent substrate or product inhibition that can occur at high concentrations, thereby maintaining optimal substrate levels for continuous product generation. Fed-batch fermentation can lead to higher product concentrations and a reduction in overall fermentation time.[10]
- **Continuous Fermentation:** In continuous systems, both the medium and inoculum are continuously fed into the bioreactor, while the culture broth is simultaneously withdrawn at the same rate to maintain a constant volume. This approach reduces downtime between batches, but it necessitates stringent aseptic conditions to prevent contamination over prolonged operation periods.[39]

Process Optimization Parameters:

Maximizing biosurfactant production requires meticulous control and optimization of various physical and nutritional parameters.[3]

- **Nutrient Sources:**
  - **Carbon Sources:** These are paramount for microbial growth and the synthesis of biosurfactants. Carbon sources can be broadly categorized into hydrocarbons, oils and fats, or carbohydrates.[60] Examples of effective carbon sources include glucose, sucrose, lactose, maltose, xylose, corn oil, olive oil, waste frying oil, molasses, spent wash from distilleries, soy hull hydrolysate, crude biomass carbohydrates, rice and corn distillers' dried grains with solubles (DDGS), cashew apple juice, and grape juice.[3]
  - **Nitrogen Sources:** Both organic (e.g., beef extract, peptone, yeast extract, soybean meal, corn meal) and inorganic (e.g., $(NH_4)_2SO_4$, NH_4NO_3, NH_4Cl, $NaNO_3$, urea) nitrogen sources are critical for microbial metabolism and biosurfactant synthesis.[35]
  - **Minerals and Trace Elements:** The presence and concentration of various ions, such as $ZnSO_4$, Na_2HPO_4, $CaCl_2$, $BaCl_2$, $CuSO_4$, $MgSO_4$, $MnSO_4$, and $FeCl_3$, can significantly influence biosurfactant production yields.[62]
- **Physical Parameters:** Environmental factors such as temperature (e.g., 25-42°C for *Bacillus*), pH (e.g., 6-9 for *Bacillus*), agitation rate, oxygen supply, inoculum size, and broth content are crucial for optimizing production.[3] Salinity is another critical environmental factor that must be carefully controlled.[6]

Optimization Techniques:

Statistical techniques such as Response Surface Methodology (RSM) are widely employed to design experiments and identify the optimal conditions for maximizing biosurfactant production. RSM allows for the simultaneous evaluation of multiple factors and their interactions, leading to more efficient process

optimization.[25]

The consistent emphasis on utilising renewable and low-cost substrates, such as various agricultural wastes (e.g., fruit wastes, molasses, waste frying oil, DDGS, fruit juices), for biosurfactant production highlights a clear and significant trend in the field.[3] This trend is a direct response to the high production costs of biosurfactants, which represent a major barrier to their widespread commercialization.[11] The focus on waste valorisation implies a strategic shift towards developing more economically viable and environmentally sustainable bioprocesses, aligning with the principles of a circular bioeconomy. This approach is crucial for biosurfactants to effectively compete with synthetic alternatives in the global market.

2.3. Advances in Genetic and Metabolic Engineering for Enhanced Production

Cutting-edge biotechnological approaches, including synthetic biology and metabolic engineering, are increasingly vital for optimizing biosurfactant production.[8] These advanced strategies aim not only to increase overall yields but also to tailor the specific characteristics of biosurfactants for diverse applications.

Strategies:

- **Strain Improvement:** Identifying and utilizing hyper-producing microorganisms is a foundational strategy for enhancing biosurfactant yields.[9]
- **Genetic Manipulation of Biosynthesis Pathways:** This involves directly modifying the microbial pathways and genetics responsible for biosurfactant synthesis. Techniques include targeted gene disruption and promoter optimization to enhance gene expression.[17]
- **Heterologous Expression Systems:** Creating synthetic biosurfactant pathways in non-native host organisms, such as *Escherichia coli*, can circumvent the complex regulatory networks present in wild-type producers. This approach facilitates the production of tailor-made biosurfactants with specific properties.[17]
- **CRISPR-Cas9 Applications:** This gene-editing technology offers precise tools for genetic manipulation, enabling targeted gene disruption and promoter optimization to significantly enhance biosurfactant production efficiency.[12]
- **Optimizing Precursor Pathways:** Enhancing the intracellular supply of precursor molecules, such as amino acids and fatty acids, is achieved by increasing the abundance and activity of associated biosynthetic enzymes. This ensures sufficient building blocks for biosurfactant assembly.[47]
- **Molecular Regulatory Networks and Tolerance Mechanisms:** Manipulating quorum sensing systems and improving the production strain's tolerance to high concentrations of the biosurfactant product can prevent negative feedback effects and maximize yield.[47]

Case Studies and Yield Improvements:

Significant progress has been made in enhancing biosurfactant production yields through these engineering approaches:

- ***Bacillus subtilis* (Surfactin):** Initial surfactin yields were low (0.05-0.1 g/L). However, through strategies like continuous foam removal (0.7-0.8 g/L), iron addition (3.5 g/L), oxygen limitation (7.0 g/L), and advanced genetic code expansion in fed-batch bioreactor processes, yields have reached up to 10.8 g/L.[46] Utilizing alternative carbon sources like cashew apple juice and grape juice has also yielded impressive concentrations of 3.65 g/L and 3.16 g/L, respectively.[17]
- ***Pseudomonas aeruginosa* (Rhamnolipids):** Reported rhamnolipid titers have varied widely, ranging from 0.5 g/L to a remarkable 78 g/L, depending on the carbohydrate source and fermentation type.[31]

Overexpression of the *rhlAB* genes has been shown to significantly enhance rhamnolipid production.[21]

- ***Candida bombicola* (*Starmerella bombicola*) (Sophorolipids):** Production has been improved with a combination of glucose and corn oil.[37] Genetic engineering has enabled the production of specific medium-chain sophorolipids.[38] Utilizing oil refinery wastes, yields of 18.14-46.1 g/L have been achieved, and using rice/corn DDGS, yields of 17.81-19.27 g/L have bee n reported.[41]
- **Mannosylerythritol Lipids (MELs):** Process optimization has led to high product concentrations, with MELs reaching 50 g/L and cellobiose lipids exceeding 20 g/L.[60]

The advancements in genetic and metabolic engineering are enabling not just increased overall yield but also the precise customisation of biosurfactant characteristics.[6] This represents a significant shift from merely producing what the wild-type organism naturally synthesises to designing biosurfactants with specific desired properties, such as tailored chain lengths, optimised polarity, improved stability, or novel functionalities.[19,52] This implies a future where biosurfactants can be engineered with high precision to meet the stringent requirements of various industries, including the highly demanding medical and pharmaceutical sectors. Such precision engineering is key to overcoming existing performance limitations and expanding the market segments for biosurfactants, ultimately unlocking their full potential[14].

3. Isolation and Purification of Biosurfactants

3.1. Overview of Common Downstream Processing Methods

Downstream processing (DSP), which encompasses the isolation and purification of biosurfactants from fermentation broths, represents a critical and often the most expensive stage in the overall production pipeline. It is estimated that DSP can account for a substantial portion, typically 60-70%, of the total accrued production costs.[60] The efficiency and sterility of these purification procedures are paramount, particularly when biosurfactants are intended for sensitive applications such as those in the medical and pharmaceutical fields, where product purity directly impacts safety and efficacy.[31] The high cost associated with DSP is a major economic bottleneck, directly impeding the commercial viability and widespread adoption of biosurfactants. This implies that current purification methods are often inefficient or costly when scaled up. Therefore, there is a critical need for innovation in developing more efficient, integrated, and cost-effective recovery systems. Research focusing on novel separation technologies or optimizing existing ones to reduce energy consumption, chemical use, and waste generation is paramount to making biosurfactants truly competitive in the global market.

Table 3: Biosurfactant Production Yields by Microorganisms and Optimization Strategies

Microbes	Biosurfactant	Optimization Strategy	Reported Yield	Reference
Bacillus subtilis	Surfactin	Initial production	0.05-0.1 g/L	46
		Continuous foam removal	0.7-0.8 g/L	46
		Iron addition	3.5 g/L	46
		Oxygen limitation	7.0 g/L	46
		Genetic code expansion	10.8 g/L	47

		(fed-batch)		
		Apple juice as carbon source	3.65 g/L	[17]
		Grape-juice carbon source	3.16 g/L	[17]
Pseudomonas aeruginosa	Rhamnolipid	Varying sugar source & fermentation type	0.5-78 g/L	[31]
		Overexpression of *rhlAB* genes	Enhanced production	[76]
Candida bombicola	Sophorolipid	Glucose and corn oil	Improved production	[37]
		Genetic engineering (medium-chain SLs)	Improved production	[38]
		Oil refinery wastes	18.14-46.1 g/L	[41]
		Rice DDGS	17.81 g/L	[69]
		Corn DDGS	19.27 g/L	[69]
Moesziomyces aphidis	MELs	Process optimization	50 g/L	[67]

3.2. Principles, Advantages, and Disadvantages of Key Techniques

Several methods are employed for the isolation and purification of biosurfactants, each with distinct principles, benefits, and limitations:

- **Acidic Precipitation:**
  - **Principle:** This method leverages the change in solubility of biosurfactants, particularly anionic types, at low pH. By acidifying the culture broth, typically to pH 2, the biosurfactant molecules lose their charge and precipitate out of the solution due to reduced electrostatic repulsion and increased hydrophobic interactions.[14]
  - **Advantages:** It is a relatively simple and low-cost technique for the initial crude extraction of biosurfactants from the fermentation broth.[14]
  - **Disadvantages:** A significant drawback is the co-precipitation of other cellular components, such as proteins and residual media components, leading to a crude product that necessitates further purification steps to achieve the desired purity.[18]
- **Solvent Extraction:**
  - **Principle:** This technique relies on the differential solubility of biosurfactants in various organic solvents. Following acidic precipitation, the biosurfactant-rich precipitate is typically dissolved in a

suitable solvent mixture, such as chloroform/methanol (often in a 3:1 v/v ratio), to selectively extract the compounds of interest.[50]

- **Advantages:** It is an effective method for isolating biosurfactants from the acidified supernatant, yielding a more concentrated extract compared to crude precipitation.[11]
- **Disadvantages:** A major concern is the involvement of potentially toxic and volatile organic solvents, which raise environmental and safety issues. Furthermore, this method requires subsequent solvent recovery and proper disposal, adding to the overall cost and complexity of the purification process.

- **Adsorption Chromatography:**
- **Principle:** Adsorption chromatography separates biosurfactants based on their differential affinity for a solid adsorbent material (stationary phase), such as silica gel.[19] Molecules in a mobile phase are adsorbed onto the solid surface and subsequently desorbed at varying rates, allowing for their separation based on their interaction strengths with the adsorbent.[12]
- **Advantages:** This is a versatile technique that is relatively simple to implement and cost-effective. It is suitable for separating a broad range of compounds, including both non-polar and polar biosurfactants, and can achieve good separation results, particularly for complex mixtures. High purity levels can be attained, with critical micellar concentration (CMC) values as low as 0.15 mg/L reported for purified fractions.[11]
- **Disadvantages:** There is a potential for irreversible adsorption of some sample molecules onto the stationary phase, which can lead to sample loss and reduced recovery. The separation efficiency is highly sensitive to various operational parameters, including the choice of adsorbent, solvent polarity, pH, ionic strength, column dimensions, temperature, and flow rate, necessitating careful optimization and stringent control.[33]

- **Foam Fractionation:**
- **Principle:** Foam fractionation is an environmentally friendly technique that capitalizes on the inherent surface-active properties of biosurfactants. Gas bubbles are sparged through the biosurfactant-containing solution, and biosurfactant molecules preferentially adsorb at the gas-liquid interface of these bubbles. This selective adsorption leads to the formation of a stable foam that is significantly enriched with the biosurfactant. The foam can then be collected and subsequently collapsed to recover the purified product.[11]
- **Advantages:** This method is characterized by its simplicity, environmental compatibility, and low energy consumption.[24] It offers high selectivity for surface-active compounds, enabling high recovery rates (exceeding 90%) and substantial enrichment (up to a 50-fold increase in concentration). Furthermore, it can be seamlessly integrated into fermentation processes for continuous biosurfactant collection, as has been demonstrated with cellobiose lipids.[67]
- **Disadvantages:** Its primary limitation is its effectiveness only for compounds that exhibit strong surface activity and readily form stable foams. Its applicability may be limited for biosurfactants with very low surface activity or those that do not readily form stable foams.

- **Membrane Separation (e.g., Ultrafiltration):**
- **Principle:** Membrane separation techniques, such as ultrafiltration (UF), employ semi-permeable membranes to separate molecules based on principles of size exclusion, solution diffusion, and solute-membrane affinity.[56] UF membranes are capable of filtering particles between 0.1 and 1μ in size and retaining larger impurities ranging from 0.005 to 10 microns, thereby effectively separating macromolecules like biosurfactants from smaller molecules or cellular debris.[58]

- **Advantages:** This method operates at normal temperatures, which prevents thermal degradation of heat-sensitive biosurfactants. It typically requires no chemical reagents, making it an energy-saving and environmentally friendly separation technology.[58] It offers high separation efficiency, low energy consumption, and a long service life, coupled with relatively simple operation and maintenance.[58] Moreover, it can retain all microorganisms, allowing for the maintenance of high microbial concentrations in bioreactors, which can be advantageous for continuous production systems.
- **Disadvantages:** A primary challenge is membrane fouling, which is the accumulation of particles on the membrane surface. Fouling can significantly decrease separation efficiency and increase maintenance requirements. Other potential operational faults include broken membrane fibers and seal ring leakage.[27] The initial capital investment for membranes can also be substantial. Furthermore, ultrafiltration may not be as effective for removing dissolved salts or very small contaminants, often necessitating complementary purification processes to achieve high purity.

4. Medical and Health Applications of Biosurfactants

The distinctive properties of biosurfactants, including their inherent low toxicity, ready biodegradability, and high biocompatibility, render them exceptionally attractive for a wide array of medical and health-related applications.[4] These attributes position them as a sustainable and often superior alternative to synthetic compounds in sensitive healthcare sectors.

4.1. Antimicrobial and Anti-biofilm Activities

Biosurfactants exhibit potent and broad-spectrum antimicrobial activities, encompassing antibacterial, antifungal, and antiviral effects.[3]

The primary mechanism underlying their antimicrobial action involves the permeabilization and disruption of microbial cell membranes and cell walls. This non-specific targeting of fundamental cellular structures can lead to pore formation, subsequent leakage of intracellular material, and ultimately, cell lysis.[5] For enveloped viruses, biosurfactants can damage or dissolve the viral envelope, thereby hindering the virus's ability to penetrate host cells and replicate.[16] This relatively non-specific targeting of fundamental cellular structures directly explains their broad-spectrum activity against a wide range of bacteria, fungi, and even enveloped viruses. A critical implication of this mechanism is that it makes it inherently challenging for microorganisms to develop resistance, unlike with many conventional antibiotics that target specific metabolic pathways. This positions biosurfactants as a highly promising strategy in the ongoing fight against emerging multidrug-resistant pathogens, offering a potential solution in the "post-antibiotic era".

Specific examples of antimicrobial activity include:

- **Rhamnolipids:** These have demonstrated activity against various bacteria such as *Serratia marcescens*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Bacillus subtilis*, with minimum inhibitory concentrations (MICs) typically ranging from 0.5 µg/mL to 32 µg/mL. They also exhibit activity against fungi like *Fusarium solani* and *Penicillium funiculosum*, with MICs reported at 75 µg/mL and 16 µg/mL, respectively.[20]
- **Surfactin:** This lipopeptide possesses potent antimicrobial properties against a wide range of bacteria, fungi, viruses, and mycoplasma. It has shown significant inhibitory effects against *Clostridium difficile* (MIC: 0.75 µg/mL) and is effective against *Bacillus cereus* and *Staphylococcus aureus*.[24]
- **Sophorolipids:** These glycolipids exhibit antibacterial activity against both Gram-negative (*E. coli*) and Gram-positive (*B. subtilis*) pathogens.[2]

Beyond direct antimicrobial effects, biosurfactants are powerful anti-biofilm agents, capable of preventing biofilm formation, disrupting established biofilms, and reducing microbial adhesion to surfaces.Their mechanism involves interfering with cell adhesion by altering cell surface hydrophobicity and disrupting membrane functions. They can also act as biodispersants, actively promoting the detachment and dispersal of cells from preformed biofilms.[10]

Notable examples of anti-biofilm efficacy include:

Rhamnolipids: Long-chain rhamnolipids isolated from *Burkholderia thailandensis* can inhibit biofilm formation by 50-90%. *Pseudomonas aeruginosa* rhamnolipids have been shown to disrupt preformed biofilms by 58.5% and effectively remove *Bordetella bronchiseptica* iofilms.[10]

Surfactin: This biosurfactant inhibits *Enterococcus faecalis* biofilm formation by interfering with pilus and exopolysaccharide biosynthesis, leading to reduced bacterial attachment and thinning of existingbiofilms.[12] *Bacillus subtilis* surfactin can disrupt preformed biofilms by an impressive 95.9%.

Lipopeptides: A lipopeptide from *Bacillus amyloliquefaciens* demonstrated significant inhibition of biofilms (96-99%) and could disperse preformed biofilms by up to 95.9%.[49] A synergistic effect has also been observed where biosurfactants can increase the efficacy of conventional antibiotics when combined, enhancing their action by 25-50%.

4.2. Role in Drug Delivery Systems

Biosurfactants play a crucial role in enhancing drug delivery capabilities by improving drug stability, solubility, and overall bioavailability, and can also facilitate more controlled release of therapeutic agents.[5, 63-36] Their amphiphilic nature enables them to form various self-aggregating nanostructures, such as micelles, microemulsions, liposomes, niosomes, cubosomes, and hexosomes.[17] These structures are highly effective in encapsulating and solubilizing both hydrophobic and hydrophilic drugs, protecting them from degradation and facilitating their transport within biological systems. Furthermore, glycolipid sugar chains can be strategically modified to target specific cells by interacting with carbohydrate-binding proteins on cell surfaces, enabling highly precise and targeted drug delivery.[27]

The remarkable ability of biosurfactants to self-assemble into diverse nanostructures (e.g., micelles, liposomes, niosomes, microemulsions) is a direct consequence of their amphiphilic nature. This property is not merely an interesting characteristic but profoundly influences their immense potential as versatile nanocarriers for drug delivery. Unlike traditional carriers, biosurfactant-based systems offer distinct advantages, including enhanced drug loading capacity, improved bioavailability, thermodynamic stability, and the possibility of targeted delivery (e.g., through specific sugar moieties on glycolipids). This makes them a promising solution for overcoming the limitations of conventional drug formulations, particularly for poorly soluble drugs or those requiring precise cellular targeting.[53] This positions biosurfactants as key players in the future of nanomedicine and pharmaceutical innovation.

Examples of their application in drug delivery include:

- **Glycolipids and Lipopeptides:** These classes are commonly utilized in biosurfactant-based microemulsion drug delivery systems, enabling various administration routes such as topical, oral, nasal, ocular, and intravenous applications.
- **Sophorolipid-based Nanoparticles (SLNPs):** SLNPs have demonstrated the ability to enhance the stability, biocompatibility, bioavailability, pharmacokinetics, and therapeutic efficacy of anticancer drugs, such as doxorubicin hydrochloride, when integrated into nanoparticle formulations.
- **Rhamnolipids:** These biosurfactants are employed as microemulsion stabilizers for various nanoparticles, including nickel oxide and silver nanoparticles, in advanced drug delivery systems.

- **β-sitosterol β-D-glucoside biosurfactant:** A liposome vector complexed with DNA using this specific biosurfactant was successfully utilized for herpes simplex virus thymidine kinase gene therapy, showcasing their potential in gene delivery.[32]

4.3. Immunomodulatory Effects

Biosurfactants possess significant immunomodulatory capabilities, allowing them to influence immune responses through direct interaction with immune cells. They can promote antigen presentation, modulate the activation state of immune cells, and facilitate crucial interactions between different immune cell types.[3] Furthermore, certain biosurfactants can act as potent immunological adjuvants, enhancing the body's immune response to vaccines or other antigens. [27] High molecular weight biosurfactants, such as lipopolysaccharides and other polysaccharide-based compounds, are particularly recognized for their immunomodulatory effects.[3] For instance, emulsan, an acylated polysaccharide, has been shown to activate macrophages in a dose-dependent manner, with its adjuvant activity being dependent on the specific fatty acid side chains decorating its polysaccharide backbone.[56] Lipopeptides like 'iturin' are acknowledged as non-toxic and non-pyrogenic immunological adjuvants.[27] Some biosurfactants, such as Mannosylerythritol Lipids (MELs), can influence cell differentiation and induce apoptosis in tumor cells, suggesting a role in immune surveillance or direct cellular modulation relevant to cancer therapy.[11] Rhamnolipids have also been demonstrated to regulate host immune function, further highlighting their diverse immunological roles.[11]

The documented immunomodulatory properties of various biosurfactants, particularly high molecular weight types (e.g., emulsan) and specific glycolipids/lipopeptides (e.g., MELs, iturin), represent a significant connection between their surface-active nature and their biological impact. This extends beyond simple antimicrobial action. This implies a substantial potential for biosurfactants in advanced therapeutic applications, such as enhancing vaccine efficacy as adjuvants or directly modulating immune responses in various disease states, including chronic inflammatory conditions or cancer. This multi-faceted interaction with biological systems positions them as promising candidates for developing novel immune-therapeutics.

4.4. Diagnostic and Therapeutic Potential

Biosurfactants hold considerable promise in both diagnostic and therapeutic applications within the medical field.

Therapeutic Applications:

- **Anticancer Activity:** Biosurfactants can induce tumor cell differentiation or death through various mechanisms. These include activating specific enzyme pathways, affecting mitochondrial function, regulating the cell cycle, increasing intracellular reactive oxygen species (ROS) levels, and triggering apoptosis.[11] Examples include sophorolipids, which effectively inhibit the viability of various cancer types, such as breast, lung, liver, cervical, and colon cancers.[34] Surfactin has demonstrated anticancer activity against breast cancer cell lines (MCF-7, T47D), colon cancer cells (LoVo, HCT-15, HT-29), and human K562 leukemia cells.[34] Mannosylerythritol lipids (MELs) have also been implicated in inducing growth arrest and apoptosis in tumor cells.[10]
- **Wound Healing and Tissue Repair:** Biosurfactants can promote wound healing and tissue repair by endorsing cellular migration and proliferation and fostering tissue remodeling, while also influencing the inflammatory phase of wound healing.[21]
- **Antiviral Activity:** Beyond their ability to damage or dissolve viral envelopes, biosurfactants exhibit specific antiviral activities. Rhamnolipids, for example, have shown activity against HSV-1 and

SARS-CoV-2.[11] Surfactin has demonstrated antiviral properties against human immunodeficiency virus 1 (HIV-1) and herpes simplex virus 1 (HSV-1).[55]

Diagnostic Applications:

Biosurfactants can serve as modifiers of functional ingredients, broadening their utility within the sphere of drug diagnosis.[8] Their ability to interact with various interfaces and biomolecules makes them valuable tools in diagnostic assays and formulations.

Comparison to Synthetic Surfactants in Medical Applications:

While biosurfactants offer clear advantages in terms of safety, biodegradability, and specific biological activities crucial for medical applications, their high production cost and often lower yields compared to synthetic counterparts remain significant barriers to widespread commercial adoption. This highlights a fundamental trade-off between the superior environmental and health profiles of biosurfactants and their economic competitiveness. For biosurfactants to truly revolutionize the medical field, ongoing research must focus not only on enhancing their efficacy and expanding their applications but critically on developing more cost-effective and scalable production and purification technologies. This economic viability is paramount for translating laboratory findings into accessible therapeutic and diagnostic tools.

- **Cost:** Biosurfactants are generally more expensive to produce than synthetic surfactants. For instance, synthetic surfactants may cost around US$ 4 per kg, whereas biosurfactants can range from US$ 34 per kg. Sophorolipids, even when produced in large quantities, are sold for $2-5 per kg, compared to synthetic alkyl polyglycoside surfactants at approximately $1 per kg. Rhamnolipids have been reported at costs ranging from $5 per kg to $150 per gram, while their synthetic counterparts might cost $10-20 per kg. The high production cost, largely due to expensive raw materials and complex processes, is a significant challenge hindering their market acceptance.
- **Yield:** Biosurfactant production yields are often variable and historically lower than those of synthetic surfactants.[11] However, significant improvements have been achieved through optimization and genetic engineering. For example, surfactin yields have increased to 10.8 g/L, rhamnolipids to 78 g/L, sophorolipids to 46.1 g/L, and MELs to 50 g/L.[17] Despite these advances, the yield per liter still needs to be competitive for broad industrial adoption.[16]
- **Toxicity and Biodegradability:** Biosurfactants are superior in terms of environmental and health safety. They exhibit significantly lower toxicity (e.g., glycolipid biosurfactants are 50% less toxic than synthetic surfactants like Tween 80; rhamnolipids and emulsan are 10 times less toxic than chemical dispersants like Corexit).[11] Their natural origin contributes to their minimal harmful effects.[11] Furthermore, biosurfactants are highly biodegradable; for instance, rhamnolipids achieved 74% degradation in 10 days under aerobic conditions, compared to 47.1% for Triton X-100.[11] Their high biocompatibility also makes them suitable for medical applications.[11]
- **Stability:** Biosurfactants generally demonstrate stable activity across a wide range of temperatures, pH levels, and salinities.[10] However, some, like surfactin, may precipitate at very low pH values.[16]
- **Specificity:** Unlike many synthetic surfactants, biosurfactants possess complex organic molecules with specific functionalities. This allows for highly targeted or site-specific actions, which is particularly beneficial for developing tailored cosmetics and diverse pharmaceutical applications.[11]

Conclusion

Biosurfactants, with their inherent amphiphilic structure, offer a compelling and sustainable alternative to conventional synthetic surfactants across a wide range of industries, particularly in the medical and

healthcare sectors. Their diverse classification, based on molecular weight and chemical composition, includes glycolipids, lipopeptides, phospholipids, and polymeric compounds, highlighting a direct correlation between their complex structures and their specific functional properties. This structural diversity allows for the precise customisation of biosurfactants for targeted applications, moving away from a one-size-fits-all approach.

Significant advancements in microbial production have been achieved through optimised fermentation strategies, including fed-batch and continuous modes, coupled with meticulous control over nutrient sources and physical parameters. The strategic shift towards utilising renewable and low-cost substrates, such as agricultural and industrial wastes, is a direct response to the economic barriers posed by high production costs, aligning with the principles of a circular bioeconomy. Furthermore, cutting-edge genetic and metabolic engineering techniques, including CRISPR-Cas9 and heterologous expression systems, are revolutionizing biosurfactant production by not only dramatically increasing yields (e.g., surfactin up to 10.8 g/L, rhamnolipids up to 78 g/L, sophorolipids up to 46.1 g/L, MELs up to 50 g/L) but also enabling the precision engineering of biosurfactants with desired characteristics for specific industrial and medical needs.

Despite these production triumphs, the downstream processing for biosurfactant isolation and purification remains a critical economic bottleneck, often accounting for the majority of the total production cost. While methods like acidic precipitation, solvent extraction, adsorption chromatography, foam fractionation, and membrane separation offer various advantages, each presents challenges that necessitate further innovation in integrated and cost-effective recovery systems.

In medical and health applications, biosurfactants demonstrate immense promise. Their broad-spectrum antimicrobial and anti-biofilm activities, primarily driven by membrane disruption, offer a potent strategy against multidrug-resistant pathogens, presenting a unique advantage over conventional antibiotics. Their ability to self-assemble into diverse nanostructures positions them as versatile nanocarriers for enhanced drug delivery, enhancing drug stability, solubility, and enabling targeted therapeutic approaches. Moreover, their immunomodulatory effects open avenues for novel immune-therapeutics and vaccine adjuvants. The documented anticancer, wound healing, and antiviral properties further solidify their therapeutic potential.

While biosurfactants possess superior safety profiles, biodegradability, and specificity compared to synthetic surfactants, their higher production costs currently limit widespread commercial adoption. The future trajectory of biosurfactants in medical applications hinges on continued innovation in bioprocess engineering and downstream purification to bridge this economic gap. As research progresses, the ability to produce tailor-made, cost-effective biosurfactants will undoubtedly unlock their full potential, establishing them as indispensable components in the quest for sustainable and advanced healthcare solutions.

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